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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>5</sup> :</b> C12N 15/11, 15/56, C07K 15/04 C12N 9/42 // (C12N 15/11 C12R 1:885)	<b>A1</b>	<b>(11) International Publication Number:</b> WO 94/04673 <b>(43) International Publication Date:</b> 3 March 1994 (03.03.94)
<b>(21) International Application Number:</b> PCT/FI93/00330 <b>(22) International Filing Date:</b> 19 August 1993 (19.08.93) <b>(30) Priority data:</b> 932,485 19 August 1992 (19.08.92) US <b>(71) Applicant:</b> OY ALKO AB [FI/FI]; Salmisaarenranta 7, FIN-00180 Helsinki (FI). <b>(72) Inventors:</b> NAKARI, Tiina, Hannele ; Kauppakartanonkuja 3 E 53, FIN-00930 Helsinki (FI). ONNELA, Maija-Leena ; Hakolahdentie 2 B 23, FIN-00200 Helsinki (FI). ILMÉN, Marja, Hannele ; Seljatie 1 A 19, FIN-00320 Helsinki (FI). NEVALAINEN, Kaisu, Milja, Helena ; 13/269-271 Maltón Road, North Epping, NSW 2121 (AU). PENTTILÄ, Merja, Elisa ; Vähäntuvantie 9 A 6, FIN-00390 Helsinki (FI).		<b>(74) Agent:</b> LÖNNQVIST, Gunnel; Oy Alko Ab, Law Department/Patents, P.O. Box 350, FIN-00101 Helsinki (FI). <b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> FUNGAL PROMOTERS ACTIVE IN THE PRESENCE OF GLUCOSE  <b>(57) Abstract</b>  A method is described for the identification and cloning of promoters that express under a defined environmental condition, such as growth in glucose medium. Using this method, five <i>Trichoderma</i> promoters capable of the high expression of operably linked coding sequences are identified, one of which is the promoter for <i>T. reesei tef1</i> . Also provided are altered <i>cbh1</i> promoters, altered so that glucose no longer represses expression from such promoter. The invention further provides vectors and hosts that utilize such promoters, and unique fungal enzyme compositions from such hosts.		

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	KspI <u>CCGCGG</u> ACTG CGCATCATGT	1740
ATCGGAAGTT GGCCGTCATC TCGGCCTTCT TGGCCACAGC TCGTGCTCAG TCGGCCTGCA		1800
CTCTCCAATC GGAGACTCAC CCGCCTCTGA CATGGCAGAA ATGCTCGTCT GGTGGCACTT		1860
GCACTCAACA GACAGGCTCC GTGGTCATCG ACGCCAAC TG GCGCTGGACT CACGCTACGA		1920
ACAGCAGCAC GAACTGCTAC GATGGCAACA CTTGGAGCTC GACCCTATGT CCTGACAACG		1980
AGACCTGCGC GAAGAACTGC TGTCTGGACG GTGCCGCCTA CGCGTCCACG TACGGAGTTA		2040
CCACGAGCGG TAACAGCCTC TCCATTGGCT TTGTCACCCA GTCTGCGCAG AAGAACGTTG		2100
GCGCTCGCCT TTACCTTATG GGCAGCGACA CGACCTACCA GGAATTCACC CTGCTTGGCA		2160
ACGAGTTCTC TTTGATGTT GATGTTTCGC AGCTGCCGTA AGTGACTTAC CATGAACCCC		2220
TGACGTATCT TCTTGTTTTG TCCAGCTGA CTGGCCAATT TAAGGTGCGG CTGGAACGGA		2280
GCTCTCTACT TCGTGCCAT GGACGCGGAT GGTGGCGTGA GCAAGTATCC CACCAACACC		2340
GCTGGCGCCA AGTACGGCAC GGGGTACTGT GACAGCCAGT GTCCCCGCGA TCTGAAGTTC		2400
ATCAATGGCC AGGCCAACGT TGAGGGCTGG GAGCCGTCAT CCAACAACGC AAACACGGGC		2460
ATTGGAGGAC ACGGAAGCTG CTGCTCTGAG ATGGATATCT GGGAGGCCAA CTCCATCTCC		2520
GAGGCTCTTA CCCCCACCC TTGCACGACT GTCGGCCAGG AGATCTGCGA GGGTGATGGG		2580
TGCGGCGGAA CTTACTCCGA TAACAGATAT GCGGGCACTT GCGATCCCGA TGGCTGCGAC		2640
TGGAACCCAT ACCGCCTGGG CAACACCAGC TTCTACGGCC CTGGCTCAAG CTTTACCCTC		2700
GATACCACCA AGAAATTGAC CGTTGTCACC CAGTCCGAGA CGTCGGGTGC CATCAACCGA		2760
TACTATGTCC AGAATGGCGT CACTTTCCAG CAGCCCAACG CCGAGCTTGG TAGTTACTCT		2820
GGCAACGAGC TCAACGATGA TTA CTGTCACA GCTGAGGAGG CAGAATTCGG CGGATCCTCT		2880
TTCTCAGACA AGGGCGGCCT GACTCAGTTC AAGAAGGCTA CCTCTGGCGG CATGGTTCTG		2940
GTCATGAGTC TGTGGGATGA TGTGAGTTG ATGGACAAAC ATGCGCGTTG ACAAAGAGTC		3000

FIG.16A

SUBSTITUTE SHEET

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<u>AAGCAGCTGA CTGAGATGTT ACAGTACTAC GCCAACATGC TGTGGCTGGA CTCCACCTAC</u>	3060
<u>CCGACAAACG AGACCTCCTC CACACCCGGT GCCGTGCGCG GAAGCTGCTC CACCAGCTCC</u>	3120
<u>GGTGTCCCTG CTCAGGTGGA ATCTCAGTCT CCCAACGCCA AGGTCACCTT CTCCAACATC</u>	3180
<u>AAGTTGGGAC CCATTGGCAG CACCGGCAAC CCTAGCGGCG GCAACCCTCC CGGCGGAAAC</u>	3240
<u>CCGCCTGGCA CCACCACCAC CCGCGGCCCA GCCACTACCA CTGGAAGCTC TCCCGGACCT</u>	3300
<u>ACCCAGTCTC ACTACGGCCA GTGCGCGGTT ATTGGCTACA GCGGCCCCAC GGTCTGCGCC</u>	3360
<u>AGCGGCACAA CTTGCCAGGT CCTGAACCCT TACTACTCTC AGTGCCTGTA AAGCTCCGTG</u>	3420
<u>CGAAAGCCTG ACGCACCAGT AGATTCTTGG TGAGCCCGTA TCATGACGGC GCGGGGAGCT</u>	3480
<u>ACATGCCCC GGGTGATTTA TTTTTTTTGT ATCTACTTCT GACCCTTTTC AAATATACGG</u>	3540

XmaI

FIG.16A(Cont.)

Title: US-10-031-496C-3

RESULT 3

AAQ58015

ID AAQ58015 standard; DNA; 1820 BP.

XX

AC AAQ58015;

XX

DT 25-MAR-2003 (revised)

DT 14-SEP-1994 (first entry)

XX

DE Sequence of plasmid pML017 which carries the shortened form of the  
DE cellobiohydrolase 1 (cbh1) promoter fused to the cbh1 gene.

XX

KW Promoter; cellobiohydrolase 1; cbh1; pML017; ss.

XX

OS Synthetic.

XX

FH Key Location/Qualifiers

FT CDS 17. .19

FT /\*tag= b

FT /label= start codon

FT misc\_feature 1773

FT /\*tag= a

FT /label= KspI-XmaI fragment

FT /note= "contains cbh1 gene"

XX

PN WO9404673-A1.

XX

PD 03-MAR-1994.

XX

PF 19-AUG-1993; 93WO-FI000330.

XX

PR 19-AUG-1992; 92US-00932485.

XX

PA (ALKO-) ALKO OY AB.

XX

PI Nakari TH, Onnela M, Ilmen MH, Nevalainen KMH, Penttilae ME;

XX

DR WPI; 1994-083192/10.

XX

PT Cloning promoters active in a desired environmental condition - used  
PT partic. for expression of genes in Trichoderma fungal hosts in glucose-  
PT contg. medium.

XX

PS Example; Fig 16A; 120pp; English.

XX

CC AAQ58015 shows the sequence of the KspI-XmaI fragment that contains the  
CC chromosomal cbh1 gene. pML017 was constructed for the production of CBH1  
CC on glucose. The plasmid pML016del15(11) was digested with the enzymes  
CC KspI and XmaI (which is 76 nucleotides downstream from the translation  
CC stop codon of the cbh1 gene. The vector part contg. the shortened cbh1  
CC promoter, the cbh1 terminator and the pBR322 sequence was ligated to the  
CC chromosomal cbh1 gene isolated as a KspI-XmaI-fragment from the  
CC chromosomal gene bank of Trichoderma reesei. The sequence of this  
CC fragment is given in FT. (Updated on 25-MAR-2003 to correct PN field.)

XX

SQ Sequence 1820 BP; 388 A; 577 C; 478 G; 377 T; 0 U; 0 Other;

Query Match 100.0%; Score 24; DB 2; Length 1820;

Best Local Similarity 100.0%; Pred. No. 0.63;

Matches 24; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

QY 1 GGCGGAAACCCGCCTGGCACCACC 24

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Db 1512 GGCGGAAACCCGCCTGGCACCACC 1535